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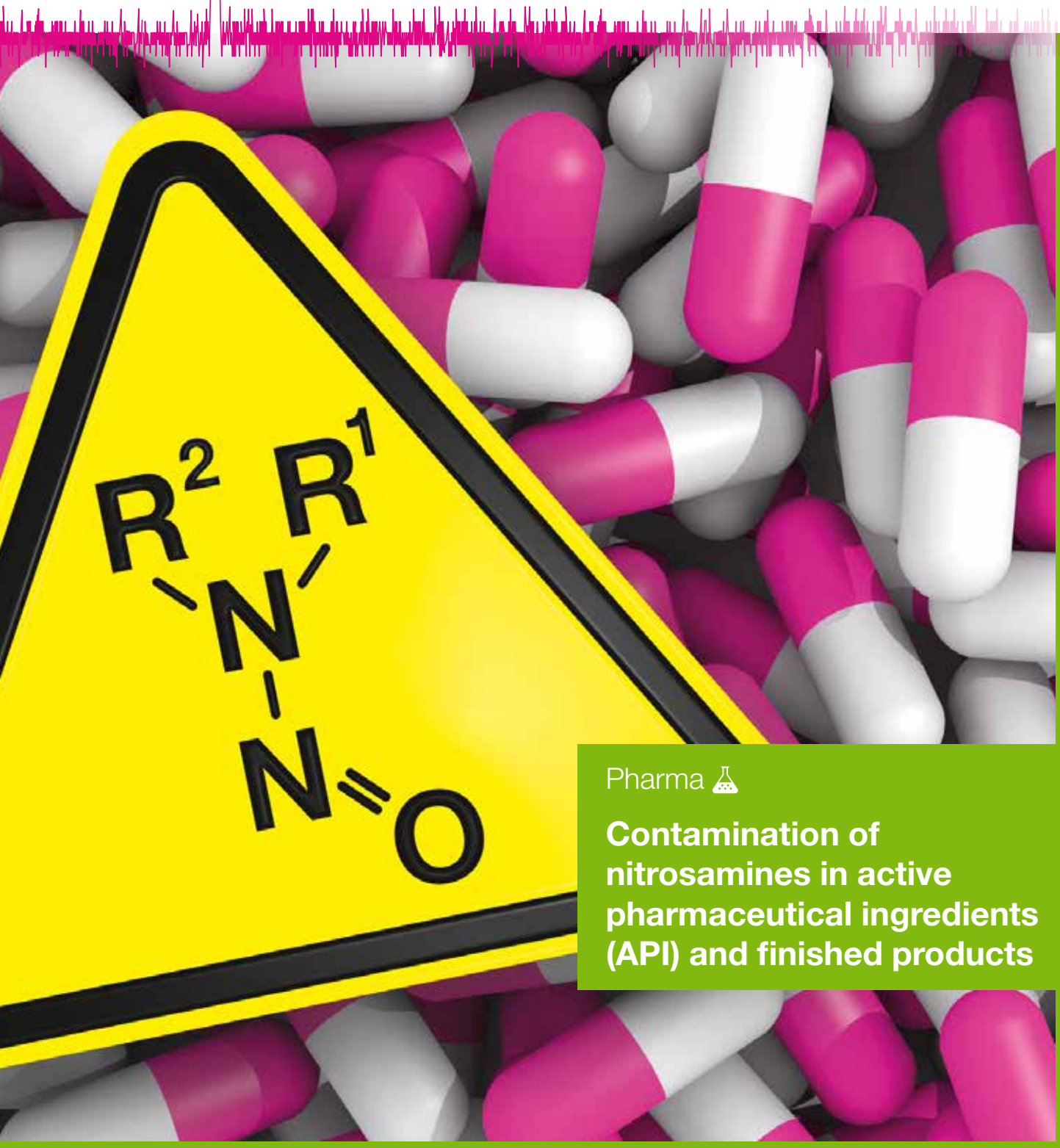
# ANALYTICS

N° 1
September 2021




Pharma

## Contamination of nitrosamines in active pharmaceutical ingredients (API) and finished products


# Contamination of nitrosamines in active pharmaceutical ingredients (API) and finished products

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## Introduction

**Since the discovery of significantly contaminated batches of the API valsartan from a Chinese manufacturer by authorities in the summer of 2018 [1], nitrosamine contamination has been discovered in several other products as well. Different manufacturers and API classes are affected by the contamination. This resulted in worldwide product recalls and strong uncertainty among pharmaceutical producers and consumers. In response to the large number of contamination cases worldwide, the European Medicines Agency (EMA) will require the exclusion of said contaminants by the marketing authorization holder (MAH) for their products [2]. In addition, the assessment of a risk analysis for each finished product is required [2]. In recent months, the FDA, Swissmedic, and other authorities have published numerous methods for detection of nitrosamines in active pharmaceutical ingredients and finished products. Each of these methods has advantages and disadvantages, but there is no unified standardized procedure.**

We at INTERLABOR Belp AG would like to support you with the analytical part of the risk assessment and present our concept for the determination of nitrosamines below.

## When should nitrosamines be investigated?

As mentioned, MAH are expected to perform a risk assessment on their product. This involves checking whether nitrosamines can be formed or introduced in the manufacturing process of the product. Possible sources of nitrosamines as well as confirmed scenarios have been summarized by EMA [4]. Swissmedic has also compiled an overview of possible nitrosamine sources [3].

The main sources of nitrosamine contamination are briefly summarized below:

- Sartan products with typical tetrazole ring
- Raw materials contaminated with nitrosamines or raw materials containing nitrites or contaminated with nitrites, which can react with existing amines
- Drinking water: Drinking water may be contaminated with NDMA as a result of water treatment, as a by-product of pesticides and disinfectants
- Solvents such as dimethylformamide (DMF), N-methylpyrrolidone (NMP) and triethylamine (TEA) can be sources of amines, which can form nitrosamines in combination with nitrating reagents during the manufacturing process
- Sodium nitrite or other nitrites in the presence of secondary or tertiary amines

## Possibilities for nitrosamine determination

If nitrosamine contamination cannot be excluded by a risk assessment, the product must be analysed for nitrosamines. There are many methods to determine nitrosamines, including simple methods such as thin layer chromatography or GC-TEA. Nowadays, nitrosamines are usually determined by Gas Chromatography or Liquid Chromatography. These techniques provide very specific and reliable results. The different Compounds can be reliably identified by retention time and specific fragmentation (MRM) based on chemical structure.

At INTERLABOR Belp AG, methods for both techniques (GC-MS/MS and LC-MS/MS) have been implemented to analyze the nitrosamines listed in **Table 1**. Swissmedic, EMA [4] and FDA [5] have established daily limits for some nitrosamines, which can be converted to a product-specific limit by means of a daily dose. The European Pharmacopoeia has also specified for sartan products that nitrosamines should be reviewed as part of a risk assessment according to the general chapter 2.5.42 (published in Ph. Eur. 10.6) [6].

## Procedure / initial risk assessment

The limit values listed above apply if **one** of the listed nitrosamines can be detected in the product. If several nitrosamines are detected, it must again be ensured that there is no risk to the ultimate consumer. In this case, the sum of the detected nitrosamines must be below the limit value of the nitrosamine with the highest potency (lowest limit).

The requirements for the method and the assurance of the thus defined limit value depend on how the nitrosamines are handled with regard to the release analysis, for example whether the nitrosamines are to be omitted from the specification or whether not every batch is to be tested (skip testing).

According to EMA, if nitrosamines are only tested once as part of the risk assessment, the method must be able to safeguard 10% of the above defined limit [4]. If only individual batches of product are to be tested after the risk assessment, the method must be able to cover 30% of the limit.

Conversely, this means that 10% of the limit should be secured during each screening in order to be able to make a statement about further steps. The risk assessment can be completed only when it is ensured that no nitrosamine contamination greater than 10% of the limit is present.

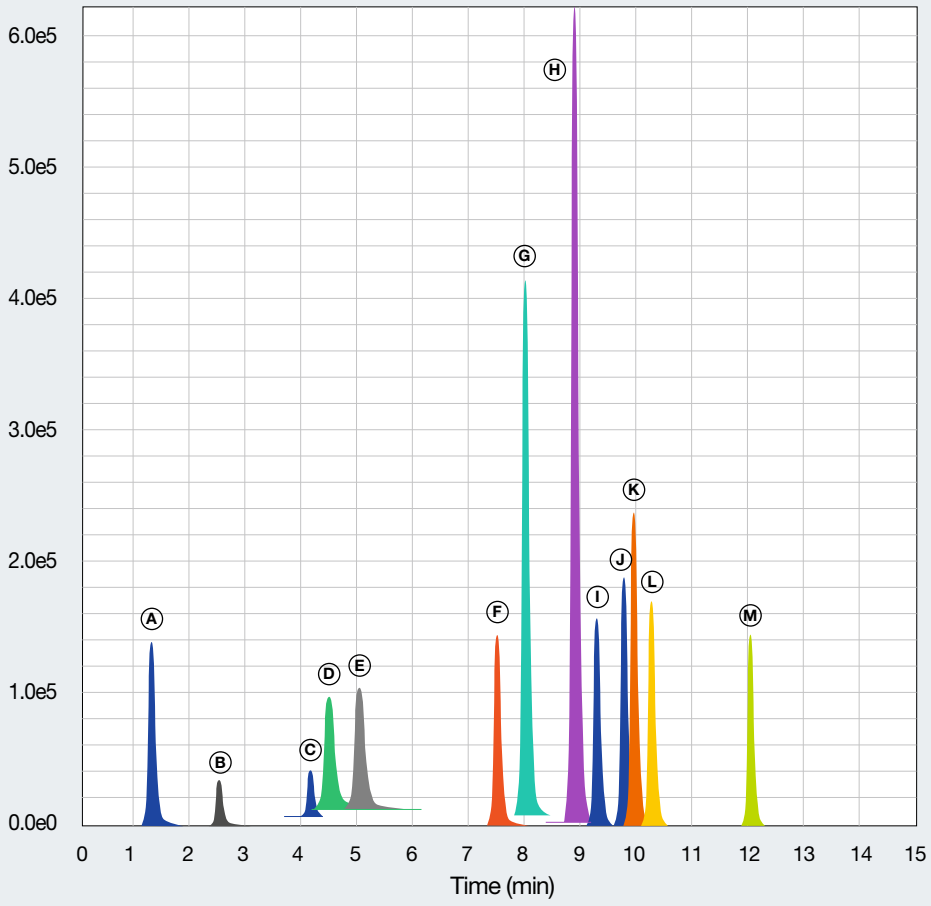
Acccording to EMA, if only 30% of the limit can be assured in a product with a complex matrix, the nitrosamines must be specified by the marketing authorization holder and regularly monitored using a validated method.

At INTERLABOR Belp AG, all nitrosamine screenings are designed in such a way that assurance of 10% of the limit can be attempted. This is not possible for all nitrosamines in all matrices. Often both techniques (GC-MS/MS and LC-MS/MS) have to be used for a complete screening.

**Table 1: Implemented nitrosamines and their limits**

| Abbreviation | Compound | CAS-Nr. | EMA [4] | FDA [5] | Swissmedic |
|---|---|---|---|---|---|
| | | | ng/day | ng/day | ng/day |
| NDMA | N-Nitrosodimethylamin | 62-75-9 | 96.0 | 96.0 | 96.0 |
| NMEA | N-Nitrosomethylethylamin | 10595-95-6 | - | - | - |
| NDEA | N-Nitrosodiethylamin | 55-18-5 | 26.5 | 26.5 | 26.5 |
| NDPA | N-Nitrosodipropylamin | 621-64-7 | - | - | - |
| NDBA | N-Nitrosodibutylamin | 924-16-3 | 26.5 | | 26.5 |
| NDIPA | N-Nitrosodiisopropylamin | 601-77-4 | 26.5 | 26.5 | 26.5 |
| NEIPA | N-Nitrosoethylisopropylamin | 16339-04-1 | 26.5 | 26.5 | 26.5 |
| NPIP | N-Nitrosopiperidine | 100-75-4 | - | - | - |
| NPYR | N-Nitrosopyrrolidine | 930-55-2 | - | - | - |
| NMBA | N-Nitrosomethylbutylamine | 7068-83-9 | - | - | - |
| NMBA | N-Nitrosomethyl butyric acid | 61445-55-4 | 96.0 | 96.0 | 96.0 |
| NMPA | N-Nitroso-N-methylanilin | 614-00-6 | - | 26.5 | 34.3 |
| MeNP | N-Nitroso-4-methylpiperazin | 16339-07-4 | 26.5 | - | 26.5 |
| NMOR | N-Nitrosomorpholine | 59-89-2 | 127 | - | - |

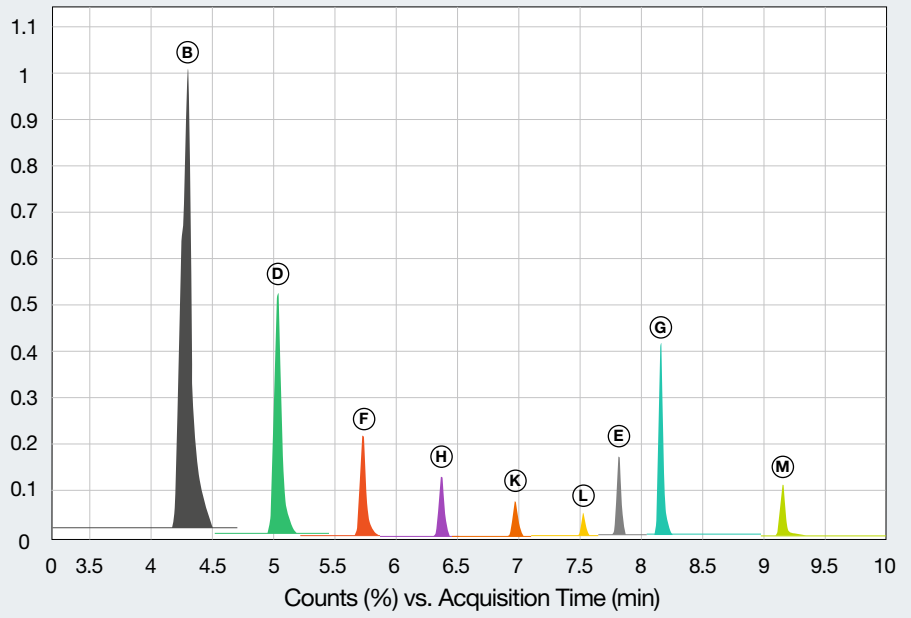
## Example chromatogram nitrosamine analytes LC-MS/MS




**Retention time (in minutes)**

A) 1.28 MeNP N-Nitroso-4-methylpiperazin (CAS 16339-07-4)
B) 2.53 NDMA N-Nitrosodimethylamin (CAS 62-75-9)
C) 4.32 NMBA N-Nitrosomethyl butyric acid (CAS 61445-55-4)
D) 4.57 NMEA N-Nitrosomethylethylamin (CAS 10595-95-6)
E) 5.03 NPYR N-Nitrosopyrrolidine (CAS 930-55-2)
F) 7.56 NDEA N-Nitrosodiethylamin (CAS 55-18-5)
G) 8.04 NPIP N-Nitrosopiperidine (CAS 100-75-4)
H) 8.91 NEIPA N-Nitrosoethylisopropylamine (CAS 16339-04-1)
I) 9.19 NMBA N-Nitrosomethylbutylamine (CAS 7068-83-9)
J) 9.71 NMPA N-Nitroso-N-methylanilin (CAS 614-00-6)
K) 9.86 NDIPA N-Nitrosodiisopropylamin CAS 601-77-4)
L) 10.16 NDPA N-Nitrosodipropylamin (CAS 621-64-7)
M) 11.99 NDBA N-Nitrosodibutylamin (CAS 924-16-3)

## Example chromatogram nitrosamine analytes GC-MS/MS




**Retention time (in minutes)**

| | | |
|---|---|---|
| B) | 4.29 | NDMA N-Nitrosodimethylamin (CAS 62-75-9) |
| D) | 5.03 | NMEA N-Nitrosomethylethylamin (CAS 10595-95-6) |
| F) | 5.72 | NDEA N-Nitrosodiethylamin (CAS 55-18-5) |
| H) | 6.36 | NEIPA N-Nitrosoethylisopropylamin (CAS 16339-04-1) |
| K) | 6.96 | NDIPA N-Nitrosodiisopropylamin CAS 601-77-4) |
| L) | 7.52 | NDPA N-Nitrosodipropylamin (CAS 621-64-7) |
| E) | 7.81 | NPYR N-Nitrosopyrrolidine (CAS 930-55-2) |
| G) | 8.15 | NPIP N-Nitrosopiperidine (CAS 100-75-4) |
| M) | 9.14 | NDBA N-Nitrosodibutylamin (CAS 924-16-3) |

## Overview

**“State of the art” screening for risk assessment.** Here we perform the determination of nitrosamines using an external calibration with internal standard correction in the range of 10% to 150% of the individually calculated limit. To verify the method, one sample each is spiked to 10%, 50% and 100% of the limit and the recovery rates are determined. For recovery rates outside the usual range of 70% to 130%, quantification is performed by standard addition.

**To perform this analysis we require the maximum daily dose and the applicable limits (EMA, FDA, Swissmedic), from which we calculate the required limit.**

This comprehensive procedure provides you with a reliable analysis result on which further steps can be based.

**Analyses within the scope of ISO 17025 or GMP .** The required effort for analyses under ISO 17025 or GMP depends strongly on the range of investigations and the required limit values. We will be pleased to provide you with a quotation for your product if you inform us of the maximum daily dose and the limit values to be applied.

## Conclusion

Recent years have shown that nitrosamines are repeatedly detected in active pharmaceutical ingredients. The publication of methods and regulations of all major drug authorities also show that there is a major risk in the manufacturing process. Due to the well-founded risk assessment and sensitive as well as selective measuring methods, the potential of nitrosamine contamination can already be determined for many manufacturing processes and products. For all products where a risk cannot be excluded, modern, safe and selective measurement techniques are available. INTERLABOR will be pleased to support you in this. □

## References


*[1] https://www.ema.europa.eu/en/human-regulatory/post-authorisation/referral-procedures/nitrosamine-impurities*

*[2] https://www.ema.europa.eu/en/news/ema-advises- companies-steps-take-avoid-nitrosamines-human-medicines*

*[3] www.swissmedic.ch "Checkliste Risikoabklärung N-Nitrosaminverunreinigung" (15.11.2019)*

*[4] EMA/409815/2020 Rev.4 "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products"*

*[5] FDA document "Control of Nitrosamine Impurities in Human Drugs – Guidance for Industry" (February 2021-Revision 1)*

*[6] https://www.edqm.eu/en/news/ph-eur-commission-adopts-new-general-chapter-analysis-n-nitrosamine-impurities (02.06.2021)*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